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Review

Assessing the Impact of Ozone on Forest Trees in An Integrative Perspective: Are Foliar Visible Symptoms Suitable Predictors for Growth Reduction? A Critical Review

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Abstract: Plant growth reduction (GR) is the most widely accepted damage parameter to assess the sensitivity of trees to tropospheric ozone (O_3) pollution since it integrates different physiological processes leading to loss of photosynthetic activity and distraction of metabolic resources from growth to defense, repair, and recovery pathways. Because of the intrinsic difficulty to assess the actual O_3 risk assessment for forests in field conditions, foliar visible symptoms (FVS) induced by O_3 have been proposed as a proxy to estimate possible GR in forest trees. The rationale for this assumption is that the onset of FVS implies a reduction of the photosynthetic capacity of plants. In this review, we show that GR and FVS can be the consequences of independent physiological pathways involving different response mechanisms that can cause both FVS without GR and GR without FVS. The onset of FVS may not lead necessarily to significant GR at plant level for several reasons, including the rise of compensatory photosynthesis, the time lag between growth processes and the accumulation of critical O_3 dose, and the negligible effect of a modest amount of injured leaves. Plant GR, on the other hand, may be induced by different physiological mechanisms not necessarily related to FVS, such as stomatal closure (i.e., carbon starvation) to avoid or reduce O_3 uptake, and the increase of respiratory processes for the production of metabolic defense compounds. Growth reduction and FVS can be interpreted as different strategies for the acclimation of plants to a stressful environment, and do not mean necessarily damage. Growth reduction (without FVS) seems to prevail in species adapted to limiting environmental conditions, that avoid loss and replacement of injured leaves because of the high metabolic cost of their production; conversely, FVS manifestation (without GR) and the replacement of injured leaves is more common in species adapted to environments with low-stress levels, since they can benefit from a rapid foliar turnover to compensate for the decreased rate of photosynthesis of the whole plant.

Keywords: ozone; forest trees; foliar visible symptoms; growth reduction; field surveys; controlled conditions experiments

1. Introduction

Sixty years ago Todd [1] described effectively the effects of tropospheric ozone (O_3) on plants in the Los Angeles region: "Damage to plants from pollutants such as those present in polluted Los Angeles air may be manifested in at least two primary ways: first, as visible oxidant damage to the leaves of susceptible plants, where it is apparent that leaf cells have been killed; and second, as a

decrease in plant growth not accompanied by visible injury". This dichotomy (foliar visible symptoms, FVS vs. growth reduction, GR) produced, in the subsequent decades, two different approaches in assessing and measuring the sensitivity of plants to O_3. Consequently, various working methods have been adopted to determine the critical levels of O_3 for plant protection [2], intended as the maximum acceptable threshold for protecting vegetation against significant adverse effects.

It is generally accepted that the responses of plants to O_3 do not depend on the ambient concentration of this pollutant (i.e., the level of exposure), but most likely on the dose absorbed by leaves through stomata, calculated as cumulated O_3 stomatal flux or phytotoxic ozone dose (POD) above a certain threshold of instantaneous flux accounting for detoxification processes [3,4]. Plants response to O_3 pressure, in fact, can be modified by the biochemical pathways aimed at neutralizing the oxidative stress induced by the reactive oxygen species generated by O_3 in the sub-stomatal cavities of leaves (detoxification) and at restoring the physiological functionality (reparation) [5].

To a general extent, however, the detrimental effects of air pollution on vegetation can be distinguished as "injury" and "damage". According to the classic definitions, *"the term 'injury' includes all plant responses that occur due to atmospheric pollution"*, while *" ... the term 'damage' includes all effects which reduce the intended value or use of the plant. The useful value—determined by economic, ecologic, and esthetic values—can be reduced through effects on growth, yield, or quality"* [6]. Plant injury is the first manifestation of an adverse effect and precedes plant damage. Growth reduction was chosen as the response indicator of the O_3 impact on trees and crops to set O_3 critical levels [7] and should be considered as a damage response variable that integrates the various physiological processes responsible for reducing the photosynthesis and biomass production rate of plants. Foliar visible symptoms should be considered as "injuries", as they consist of single or groups of dead cells in a specific plant organ (the leaf).

Relationships between O_3-induced injuries (FVS) and damage (GR) were found, in scientific studies, to be uncertain and contradictory, and are still object of debate. It is generally accepted that both manifestations are consequence of the O_3 stomatal uptake that compromise the photosynthetic apparatus and function. As a logic consequence, FVS and the consequent leaf loss (defoliation), two easily assessable indicators in field conditions, have been recently investigated and proposed as potential proxies for O_3 fluxes and, ultimately, for GR [8–10].

Most forests in Europe are exposed to O_3 pollution exceeding the established "critical levels" [11,12]; therefore, impacts on plant leaves, growth, and vitality (defined by defoliation, [13]) can be expected. However, the analysis of data of forest health condition from current monitoring programs (ICP Forests networks, and National Forest Inventories) at national and European scale, combined with measured and/or modeled meteorological conditions and O_3 levels, provided no or contrasting evidence of the above-mentioned impacts [8,14–18]. Cailleret et al. [19] conclude that the impacts of O_3 on mature forests seem to be negligible because of several confounding factors affecting the ecotoxicological response of plants, including the variability of site conditions, ecological and management characteristics of the forest stands and differences in methodological approaches.

In the present review paper, by considering experimental results of the present research group and literature findings, we aim at exploring the relationships between FVS and GR due to O_3 exposure and/or fluxes in forest tree species, and the possible mechanisms of decoupling these two different responses. We will evidence, in an integrative ecological perspective, the physiological patterns implied in the plant responses to O_3, at leaf and tree level, and the environmental factors that can affect them.

A simplified scheme of the different mechanisms and processes involved in plant response to O_3 stress and their effects at plant level in terms of FVS and GR is reported in Figure 1.

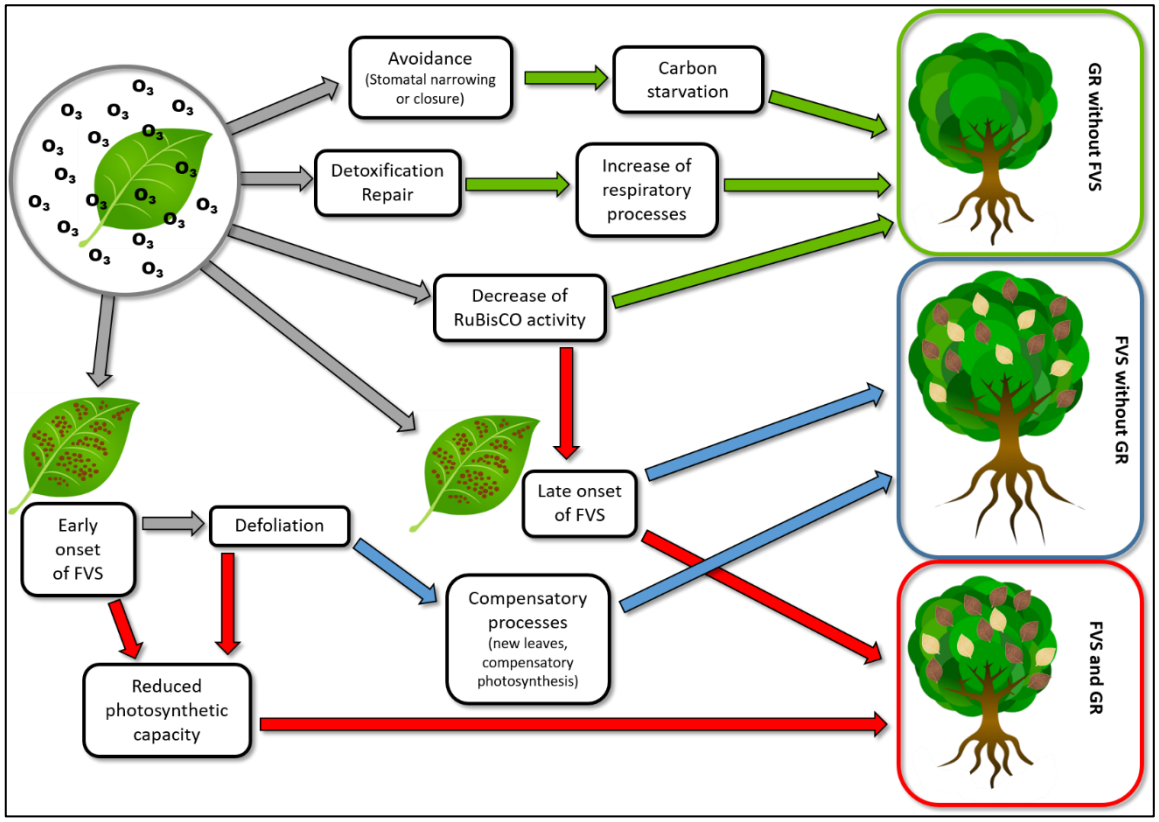

Figure 1. Relationships between the main physiological processes and mechanisms involved in plant response to O_3 stress at the leaf level, and their effects at plant level in terms of foliar visible symptoms (FVS) and/or growth reduction (GR). Green, blue, and red arrows identify different pathways leading to GR without FVS, FVS without GR, and FVS with GR, respectively.

2. Factors Implied in the Onset of Foliar Visible Symptoms

The term "foliar visible symptoms" summarizes and shows various events that occur in the leaves resulting from physiological and biochemical reactions, triggered by the ROS generated by O_3 inside the leaf, possibly interacting with other stress factors (e.g., high light, drought, nutrient deficiency) and the detoxifying and reparation capacity of the leaf tissue cells. The most typical foliar symptoms consist of the clusters of palisade mesophyll cells collapsing, thereby simulating a hypersensitive response (HR), by forming so-called stipples [20]. High-intensity sunlight radiations are considered a key factor for the onset of symptoms [21,22] because, in such conditions, stomatal activity and gas exchange rates are usually enhanced, maximizing the O3 uptake. In addition, high light intensity itself enhances the production of ROS [23] and triggers the formation of screening pigments (anthocyanins) that characterize the symptomatology of many species [24]. The sensitivity of leaves, and therefore the capacity of O_3 fluxes to produce symptoms, is variable during the life span of the leaves, and in many species, symptoms become visible at the end of the growing season when the metabolic defenses are declining [25].

In sensitive species, HR-like symptoms are tightly related to fluxes of O_3 into the leaves, but there are no linear relationships between the absorbed dose and the onset and extension of symptoms [26]. After the first appearance of symptoms, the following development and extension may follow patterns independent from the absorbed dose of O_3. The collapse of the cells adjacent to the damaged areas and ethylene production [27] within mesophyll tissue are both physiological events that, although triggered by the initial dose of O_3, increase the extension of symptoms regardless of the additional dose adsorbed [26]. Finally, leaves lose their overall functionality and no further response to O_3 can be observed. In general, we can argue that O_3 uptake is a necessary precondition to produce

FVS, but the type of visible manifestation, the timing of onset, and the successive development strictly depends on the physiological and biochemical interactions within the leaf tissues itself and the environmental conditions.

3. Factors Implied in Growth Reduction

In very sensitive tree species, with large diffusion of symptoms in the crown and widespread defoliation, the substantial quantitative reduction of the photosynthetic apparatus can lead to a significant reduction of the plant's growth rate [28].

Growth reduction without FVS, however, can be induced by different mechanisms which are more related to a reduction of the photosynthesis functionality rather than to a reduction of the available photosynthetic surface. Many studies (reviewed by Wittig et al. [29]) evidenced that photosynthetic rates are reduced after exposure to O_3, through a variety of physiological mechanisms. Fontaine et al. [30] hypothesized that O_3 contributes to the inactivation of Rubisco, so inhibiting the Calvin cycle of the photosynthetic process. Bussotti et al. [31], through chlorophyll fluorescence analysis, proposed a block of the electrons transport after the photosystem 1 (PSI), probably caused by a reduction of both amount and functionality of Rubisco within the leaf mesophyll.

A second mechanism that can lead to GR consists of the stomatal closure as a response to high levels of O_3 [32,33], so preventing O_3 uptake by leaves. This is an "avoidance" mechanism that can protect the photosynthetic machinery, but at the same time, it can also reduce the stomatal uptake of CO_2, so depressing photosynthetic rates and growth. Stomatal closure induces a "carbon starvation" like that induced by drought stress [34]. It can be stated that, in this case, it is not the level of O_3 stomatal fluxes that inhibits growth, but instead, the reduction of CO_2 entering the leaves.

The repair capacity of plants, although considered an element of resistance to O_3, may be a cause of GR because it induces the diversion of the photosynthetates [35] from growth processes to defense mechanisms. O_3-induced detoxification processes require energy for the regeneration and de-novo synthesis of antioxidants and other related chemical compounds. The decrease of photosynthetates may also be attributed to an increase of the dark respiration rate, due to an intense metabolic activity to ensure the maintenance and repair processes [35,36]. In general, it is likely that detoxification, repair, and down-regulation of Rubisco activity can lead to a change of carbon assimilation and allocation in ozone-stressed plants and to GR on a long-term time scale.

Studies on the magnitude of the relationships between GR and O_3 exposure and uptake were carried out on tree seedlings, in controlled and semi-controlled conditions, to establish the critical levels for the main representative forest species [3,37]. However, the possibility of applying the findings of these studies on mature trees, in natural forest stands, remains problematic [19], although some empirical attempts have been made [38].

4. Why Foliar Visible Symptoms and Growth Reduction Are Frequently Decoupled?

Foliar visible symptoms provoke the reduction of photosynthetic leaf surface, with a consequent expected decrease in plant growth, then in ecosystem productivity and yield (e.g., in crop, forest, and grassland systems). Several studies carried out in the past decades on both young and adult forest trees have tried to characterize the effect of O_3 considering FVS or GR. However, only a limited number of those studies analyzed both kinds of responses on the same plants (reviewed in Tables 1 and 2). Table 1 summarizes the literature, with related outcomes, of the last 35 years dealing with experimental studies in controlled conditions (i.e., greenhouses, growth chambers, open-top chambers) on both FVS and GR in forest tree species. The table contains information on the experimental setup and the most relevant findings on the effect of O_3 on FVS and GR of plants, highlighting all the situations in which FVS and GR were found to be decoupled.

Although the results of studies on O_3 effects on seedlings and adult trees are scarcely comparable [39], it is possible to identify some general patterns in the responses of plants to O_3. Many controlled conditions experiments have considered species and genera already known as O_3

sensitive (based on previous literature and observations [24,40]) such as *Populus* sp. pl. (that is the most studied genus), *Fagus sylvatica* L., *Betula pendula* Roth., *Viburnum lantana* L., and *Prunus serotina* Ehrh. When O_3 tolerant species were used (for example, *Quercus* sp. pl., an evergreen Mediterranean species), no or slight FVS were observed, but GR was detected in many cases [41–44]. Overall, in a total of 30 experiments considered (Table 1), 40 different species have been investigated (and several *Populus* hybrids) with half of them showing no simultaneous manifestation of FVS and GR in at least one experiment. For six species, there was at least one experiment reporting no effects of O_3 at all (no FVS and no GR). Only 14 of the 40 studied species showed a clear simultaneous presence of FVS and GR in at least one experiment. Nonetheless, it is important to highlight that in some experiments reporting no significant GR due to O_3, plants growth was assessed by measuring only height and stem diameter (no biomass) or by measuring only the above-ground biomass. However, it is well known that in many species, root growth is more reduced than shoot growth in response to O_3 [44–46], since O_3 can alter root physiology and reduce the belowground allocation of carbohydrates [47]. There is high variability and sometimes contradictory results between different experiments due to factors such as the genotype (clone or seed reproduced), the age of the plants, the duration and the technical characteristics of the experiments. On this latter aspect, it is worth mentioning that greenhouses and closed chambers systems may significantly reduce the UV radiation exposure of plants introducing further bias to the results of these experiments.

Table 1. Experimental studies on forest trees species which considered both foliar visible symptoms (FVS) and growth reduction (GR) due to O_3 stress. Bold underlined text highlights missing correspondence between FVS and GR in the results. Papers reporting results from the same experiment have been grouped in the same row. Studies are listed in chronological order.

Authors	Species	Plant Age	Experimental Set up	O_3 Treat. Duration	Detection of FVS	Detection of Significant GR	Notes
Hogsett et al. [48]	*Pinus elliottii*	Seedlings	O_3 fumigation chambers	16 weeks	Yes	Yes	
Wang et al. [49]	*Populus tremuloides* (O_3-tolerant and O_3-sensitive clones)	Saplings	Open-top chambers	2.5 growing seasons	Yes	Yes	
Wang et al. [50]	*Populus* hybrid; *Populus deltoides*; *Robinia pseudoacacia*	Seedlings	Open-top chambers	1 growing season	**No**	**Yes** (*Populus* hybrid)	Only the above-ground biomass of *Populus* hybrid was significantly reduced
Temple [51]	*Pinus jefferyi*; *Sequoiadendron giganteum*	2-year old seedlings	O_3 fumigation chambers	2 months for 2 growing seasons	**Yes** (*P. jefferyi*) **No** (*S. giganteum*)	**No**	
Karnosky et al. [52]	*Populus tremuloides* (various O_3 tolerant and O_3 sensitive clones)	Rooted cuttings	Open-top chambers	1 growing season	Yes	Yes	Only the O_3 sensitive clones showed FVS and GR of the above-ground biomass
Karnosky et al. [53]	*Populus tremuloides* (2 O_3 sensitive clones)	Rooted cuttings	Open-top chambers	1 growing season	**Yes**	**No**	
Pääkkönen et al. [54]	*Betula pendula*	2-year old seedlings	Open field O_3 fumigation and O_3 fumigation chambers	2 growing seasons	Yes	Yes	
Shimizu et al. [55]	*Populus* hybrids; *Abies firma*; *Quercus acutissima*; *Cryptomeria japonica*; *Viburnum odoratissimum*	2-year old seedlings; cuttings	O_3 fumigation chambers	3-4 weeks	**Yes** (*Populus* hybrids; *V. odoratissimum*) **No** (*C. japonica*; *A. firma*; *Q. acutissima*)	**Yes** (one *Populus* hybrid; *C. japonica*; *V. odoratissimum*) **No** (one *Populus* hybrid; *A. firma*; *Q. acutissima*)	
Temple and Miller [56]	*Pinus ponderosa*	Seedlings	Open-top chambers	3 growing seasons	Yes	Yes	Only stem diameter was measured
Samuelson [57]	*Prunus serotina*; *Acer rubrum*	1-year old seedlings	Open-top chambers	1 growing season	**Yes**	Yes (*P. serotina*) **No** (*A. rubrum*)	Only plant height and root/shoot ratio of *Prunus serotina* were significantly reduced
Matyssek et al. [58]	*Betula pendula*	Rooted cuttings	Open-top chambers	1 growing season	Yes	Yes	No information on the statistical significance of the GR
Karnosky et al. [59]	*Populus tremuloides*	Rooted cuttings	Open-top chambers	2 growing seasons	Yes	Yes	
Pääkkönen et al. [60]	*Betula pendula* (4 clones)	2-year old seedlings	Open field growing in 3 different sites and open field O_3 fumigation	2 growing seasons	Yes	Yes	Only plant height was significantly reduced and only after the 2nd growing season
Günthardt-Goerg et al. [61,62]	*Fagus sylvatica*; *Prunus serotina*; *Carpinus betulus*; *Fraxinus excelsior*; *Sorbus aucuparia*	Rooted cuttings; seedlings	O_3 fumigation Chambers	1 growing season	**Yes**	**No**	
Karnosky et al. [63,64]	*Populus tremuloides* (O_3-tolerant and O_3-sensitive clones)	Rooted cuttings	Open field growing in 3 sites with different ambient O_3 levels	5 growing seasons	Yes	Yes	Only volume growth was measured

Table 1. *Cont.*

Authors	Species	Plant Age	Experimental Set up	O_3 Treat. Duration	Detection of FVS	Detection of Significant GR	Notes
Inclàn et al. [65]	*Quercus ilex*; *Olea europea*; *Ceratonia siliqua*; *Arbutus unedo*	2-year old seedlings	Open-top chambers	10 months	Yes (*Q. ilex*; *A. unedo*) No (*O. europea*; *C. siliqua*)	No (*A. unedo*) Yes (*O. europea*)	Only plant height and stem diameter were measured
Yun and Laurence [66]	*Populus tremuloides* (2 clones)	Rooted cuttings	Open-top chambers	3 months	Yes	Yes	
Paludan-Müller et al. [67]	*Fagus sylvatica*	Seedlings	Open-top chambers	2 growing seasons	No	Yes	Only the root/shoot ratio showed a significant reduction
Ribas et al. [41]	*Quercus ilex*; *Ceratonia siliqua*	1-year old seedlings	Open-top chambers	2 growing seasons	No	Yes	
Ribas et al. [42]	*Quercus ilex*; *Quercus ballota*; *Ceratonia siliqua*; *Olea europea*	1-year old seedlings	Open-top chambers	2 growing seasons	No	Yes (*Q. ilex*; *Q. ballota*; *C. siliqua*) No (*O. europea*)	Only the above-ground biomass was measured
Thomas et al. [68]	*Picea abies*	2-year old seedlings	Open field O_3 fumigation	3 growing seasons	Yes	Yes	FVS and reduced shoot elongation starting from the 2nd growing season
Calatayud et al. [69]	*Acer campestre*; *Acer opalus*; *Acer monspessulanum*; *Acer pseudoplatanus*	3-year old 4-year old seedlings;	Open-top chambers	1 growing season	Yes	No	Only plant height was measured
Novak et al. [70]	*Populus nigra*; *Viburnum lantana*; *Fraxinus excelsior*	Seedings	Open-top chambers	2 growing seasons	Yes	Yes (*P. nigra*) No (*V. lantana*; *F. excelsior*)	Only ring width was measured
Novak et al. [71]	*Fagus sylvatica*; *Viburnum lantana*	2-year old seedlings	Open-top chambers	2 growing seasons	Yes	No	
Nikula et al. [72]	*Populus tremula* *Populus* hybrid	1-year old cuttings	Open field O_3 fumigation	2.5 months	Yes	No	
Gerosa et al. [73] Marzuoli et al. [26] Pollastrini et al. [74]	*Populus* hybrid; *Fagus sylvatica*; *Quercus robur*	2-year old seedlings	Open-top chambers	2 growing seasons	Yes (*Populus* hybrid; *F. sylvatica*) No (*Q. robur*)	Yes (*Populus* hybrid) No (*F. sylvatica*; *Q. robur*)	Only the above-ground biomass was measured
Calatayud et al. [43]	*Quercus ilex*; *Quercus faginea*, *Quercus robur*; *Quercus pyrenaica*	2-year old seedlings	Open-top chambers	2 growing seasons	Yes No (*Q. ilex*)	No Yes (*Q. pyrenaica*)	Only shoot/root ratio of *Q. pyrenaica* was affected. FVS were slight on *Q. robur* and *Q. faginea*
Dìaz-de-Quijano et al. [45]	*Pinus uncinata*	7-year old saplings	Open field O_3 fumigation	2 growing seasons	Yes	Yes	Only the root biomass was significantly reduced
Zhang et al. [75]	*Lyriodendron chinense*; *Liquidambar formosana*; *Cinnamomum camphora*; *Cyclobalanopsis clauca*; *Neolitsea sericea*; *Schima superba*	1-year old	Open-top chambers	6-7 weeks	Yes No (*N. sericea*)	Yes No (*N. sericea*; *C. clauca*)	
Gerosa et al. [44]	*Quercus ilex*	2-year old	Open-top chambers	1 growing season	No	Yes	

Table 2. Observational field studies on forest trees that considered both foliar visible symptoms (FVS) and growth reduction (GR). Bold underlined text highlights the missing correspondence between FVS and GR. Studies are listed in chronological order.

Authors	Species	Plant Age	Survey Methodology	GR Variables Considered	Main Results	Relationship between FVS and GR?
Peterson et al. [77]	*Pinus jeffreyi*	Mature forest	Comparison between 5 sites with symptomatic trees and 3 sites with asymptomatic trees	Growth index calculated from radial growth	Mean annual radial increment of trees with FVS was 11% less than trees at sites without injury	Yes
Peterson and Arbaugh [79]	Pinus ponderosa	Mature forest	Comparison between 5 sites with symptomatic trees and 2 sites with asymptomatic trees.	Growth index calculated from radial growth	No significant change in growth associated with FVS on trees	No
Peterson et al. [80]	*Pinus ponderosa*	Mature forest (>50 years)	Comparison between 4 stands from 7 sites with symptomatic trees and 4 stands from 7 sites with asymptomatic trees.	Radial growth	No evidence of widespread regional growth decreases during recent years, but in a few symptomatic sites, trees showed significant growth decreases	Contradictory results
Somers et al. [78]	*Prunus serotina; Liriodendron tulipifera*	Mature forest	Comparison between symptomatic and asymptomatic trees of the same species. Analysis on 30 trees for each species	Radial growth over 5- and 10-year periods	No evidence that GR in *P. serotina* was related to FVS. In *L. tulipifera* GR was significantly more intense in trees with FVS	Yes (*L. tulipifera*) No (*Prunus serotina*)
Gravano et al. [76]	*Ailanthus altissima*	3–6 years old	Comparison between 2 groups of 5 ramets, with different exposure to ambient O_3 (open field and protected below trees canopy)	Growth of the main stem, n of leaves	FVS and GR were stronger in plants of the open field site.	Yes
Vollenweider et al. [28]	*Prunus serotina*	86-year old on average	Comparison between symptomatic and asymptomatic trees in the same site	Tree diameter and basal area	Over a 31-year period, trees with FVS had 28% lower stem growth rates than asymptomatic trees	Yes

To understand what really happens in the field on trees exposed to ambient O_3, several studies have been carried out. The results of these field studies are showed in Table 2, where both FVS surveys and GR assessments (mainly radial growth assessments) were considered. Also in this case, we report the main findings and highlight the decoupling between FVS and GR.

In field studies, GR has been related to FVS on a very sensitive tree species, such as *Ailanthus altissima* (Mill.) Swingle [76], *Pinus jeffreyi* Balf. [77] and *Liriodendron tulipifera* L. [78], while contrasting results have been found on *Pinus ponderosa* Douglas ex. C. Lawson [79,80] and *Prunus serotina* [28]. In strongly polluted sites such as the surroundings of Mexico City [81] and South California mountains [82], O_3 was considered an important factor contributing to the decline of conifer forests; however, those surveys did not report data on potential relationships between FVS and GR. In an open-air fumigation system on mature trees, widespread FVS were observed on the outer, sun-exposed, leaves of *Fagus sylvatica* [83], but the GR of stem, estimated with a model during the same experiment, was attributed to stomatal narrowing or closure of the inner leaves rather to the effect of O_3 on net carbon gain [32]. Finally, although the *Populus* species and clones were generally sensitive in controlled experiments (Table 1), no FVS and/or GR was observed in a mature poplar plantation in Belgium despite a large O_3 uptake measured during the growing season [84].

The reasons FVS and GR cannot be necessarily related and coupled can be summarized as follows.

4.1. Compensatory Photosynthesis

Compensatory photosynthesis (CP) is the enhancement of the photosynthetic activity to cope with stress induced by defoliation (i.e., leaf loss) and foliar injury [85], sustaining plant growth and defense. Compensatory photosynthesis has been studied especially in relation to fungal infections and herbivorous pest attacks [86], and may occur at both single-leaf and whole-crown levels. At foliar level, CP consists in the increase of photosynthetic efficiency of the undamaged mesophyll cells, with special

reference to those surrounding the damaged ones [87]. Halitschke et al. [88] observed in tobacco leaves affected by the insect *Tupiocoris notatus* the activation of genes related to photosynthesis. According to Meyer [89] CP represents an efficient tool when foliar damage has a limited extension, and the damaged leaves are sparse in the crown. In such conditions, plant productivity and yield are maintained or increased [90]. At the whole-crown level, CP consists of the substitution of the inefficient damaged leaves with a new photosynthetic apparatus [91,92] and enhanced efficiency in the use of light in partially defoliated crowns [56].

4.2. Modest Amount of Injured Leaves

Usually, injuries affect the upper and outer parts of the crown, especially the sun-exposed leaves [93]. In some tree species, such as *Fagus sylvatica*, the prevalent role of the sun-exposed leaves is to protect the inner foliage, most devoted to photosynthesis, from high light intensity radiation, and the consequent photoinhibition [94]. In a mature tree, the number of injured leaves within the crown may be therefore very small, and their negative effect on the total photosynthesis of the tree can be negligible.

4.3. Late Season Onset of Foliar Visible Symptoms (After the End of the Growth Process of Plant)

Except for the most sensitive *taxa* (for example, poplar clones [70]), FVS usually appear in the late summer [25,95]. There is likely an interaction during the growing season between O_3 stress, the exposure to high solar intensity radiation, and the onset of the leaf senescence process, which leads to the decline of the photosynthesis and therefore to the decline of biochemical defense [96]. Plants in which the growth and reproductive processes occur in the first part of the season can show the late-season onset of FVS, avoiding any significant growth reduction [25,74]. Contrarily, the plants with continuous growth over the whole season, such as poplar, are more susceptible to the reduction of biomass accumulation [70].

5. Significance of Foliar Visible Symptoms Assessment in the Field

The European program for the monitoring of forest conditions in Europe (ICP Forests) includes the assessment of O_3-induced FVS on the native vegetation in field conditions. The rationale of this activity is stated in the manual [93]: "*The assessment of ozone visible injury serves . . . as a means to estimate the potential risk for European ecosystems that are exposed to elevated ambient ozone*". In order to check the reliability of such a statement, it is necessary to define what is "risk" for forests and how to quantify it. There is agreement in literature [97] that the concept of risk is related to the probability that damage can happen, and it can be measured through numerical exposure and dose indices [98], with special reference to the O_3 fluxes entering into the leaves. In this perspective, FVS caused by O_3 have been listed among the indicators of impact of pollutants on forests in the regulations of the European Union (NEC, National Emission Ceilings, directive 2016/2284/EU, https://www.eea.europa.eu/themes/air/national-emission-ceilings/national-emission-ceilings-directive). On the other hand, GR in terms of decrease of plant biomass production has been selected within the CLRTAP [4] as the response variable for quantifying and mapping O_3 risk for vegetation based on critical levels exceedances in Europe. In this context, some studies have unsuccessfully tried to find relationships between FVS, O_3 stomatal fluxes, and GR of trees in the field [9,10,14,16,19]. In the previous paragraphs, we have indeed shown that there are no conceptual and mechanistic relationships between FVS and GR since these two manifestations in the plant follow independent patterns and can be considered as indicative of the effects of the different strategies applied by trees to cope with O_3 pollution stress. There are, however, additional reasons because the correlations between FVS and GR in field conditions are questionable.

5.1. Variability of the Symptomatology

Many FVS have low specificity [99,100] and can be more likely attributed to other factors than O_3, such as high solar radiation, early leaf senescence, or lack of nutrients. Leaf reddening and bronzing,

two of the more widespread visible manifestation considered as O_3 symptoms, are strongly dependent on high light intensity. Intense solar radiation can be able to produce foliar visible injuries also without the presence of the O_3 itself [101].

5.2. Variability of O_3 Sensitivity within the Same Species

Plants of the same species can react in different ways to the same O_3 levels of exposure and/or foliar uptake in relation to ontogenetic factors, ecological conditions, and genotypes. Epigenetic factors [102] were not fully explored, but may also be relevant.

5.3. Variability in Plant Communities

The heterogeneity of specific plant composition within a plant community and the variability in size, age and development, as well as the variability of the ecological conditions make different sites not comparable each other [16]. The absence of foliar visible symptoms does not imply an absence of O_3 pollution and an absence of O_3 risk for vegetation.

6. Discussion

Foliar visible symptoms represent a wide and heterogeneous biological aspect that includes manifestations and processes with different physiological and ecological meanings. Relationships between FVS and O_3 uptake can be verified only on standard plant material, homogeneous for genetic features and condition of cultivation, i.e., by applying a classic "bioindicator" approach [103]. In the case of trees, poplars have been proposed as model species [104,105]. In a perspective of "passive" bioindication, promising results were obtained by using one species at a limited regional scale [106]. Leaves of *Viburnum lantana* plants were found to have widespread symptoms (reddening) in sites with higher O_3 concentrations, with a response consistent across space and different years. Similar results were obtained with *Pinus cembra* L. [9] and *Pinus halepensis* Mill. [107].

The most typical foliar symptoms, HR-like, are related to high O_3 stomatal flux rates (that are, in turn, connected to high stomatal conductance and photosynthetic rates) and low defense investments. Plant species with these characteristics are also fast-growing species and have high nitrogen content and specific leaf area (SLA). In addition, they show indeterminate growth and the ability to produce new leaves for compensatory photosynthesis during the whole growing season. These features are typical of early successional plant species [108,109], or demanding species growing in an optimal condition of soil, mineral nutrition, and water supply. In such conditions, trees under stress tend to not defend their leaves and to substitute the damaged ones (to limit respiration losses) with new more efficient leaves. This is a way to sustain growth even under stressful situations. In opposite conditions, i.e., in late successional species, especially growing on low fertility soils, leaves have low SLA, and the metabolic investment per leaf is high. Trees under stress tend to defend their leaves producing antioxidant compounds and closing the stomata, so reducing their photosynthetic rate, and consequently, their growth rate. According to the universal adaptive strategy theory [110], FVS are a typical response of ruderal and competitors plant species, whereas GR without FVS may be more common in the stress-tolerant species. In other words, the strategies of resistance, recovery and resilience of forest tree species are dependent on their functional group, successional position, ecological behavior, and, ultimately, on their phylogenetic history. Only considering all these aspects, it is possible to understand the different responses of trees to O_3 and other environmental stresses.

Growth reduction, as an indicator of damage, has a clear meaning when it is referred to productive systems ("economic" damage), but does not fit properly in natural ecosystems where biodiversity conservation is the main purpose of the management. According to Körner [111], the biomass production (yield) is an agronomic concept that can be applied in productive systems with no limitation of environmental resources, but it does not apply to a plant community. In ecology, the state of a system relies on the reproductive fitness and species abundance. There are no or limited field evidences about a possible impact of O_3 on the structure and composition of forest ecosystems, as well on their internal

dynamics and the relationships between biotic and abiotic components, although a modeling approach suggests that in many eco-regions O_3 risks will persist for biodiversity at different trophic levels and for a range of ecosystem processes and feedback [112]. In such a context, tropospheric O_3 pollution should be considered as a component of global change together with increasing drought and temperature, CO_2 atmospheric concentration, nitrogen deposition, UV radiations, and so on [113]. The interactions are complex, both synergistic and antagonistic, and the contribution of each factor may vary in space and time. Potters et al. [114] suggest that a maze of interchangeable physiological and molecular processes, rather than a single pathway, is responsible for the induction of the responses of plants under different stress conditions. In this perspective, GR can be considered as a form of acclimation for plants reaching out for a new equilibrium in a changing/changed environment, rather than true damage at physiological and/or ecological level [115]. Damage may occur when the progressive harshening of the environmental factors or the recurrence of extreme events overcome the capacity of the ecosystems to maintain or to restore the ecological equilibrium.

7. Conclusions

The findings present a claim for a reconsideration of the concept of O_3 "sensitive" or "resistant" species since such species classification may change, taking into consideration different physiological responses within an ecological context. The analysis of the available literature on the relation between FVS and GR due to tropospheric O_3 stress suggests that the current knowledge and the state-of-art can be biased by different factors: (i) the experiments in controlled conditions were carried out mostly on species already supposed as sensitive (at least in relation to FVS), and these results can hardly be used for the comprehension of the processes in natural conditions; (ii) mature trees have different physiological behavior compared to seedlings, and controlled condition experiments on seedlings may be used more properly to study the impact of O_3 on tree regeneration in the gaps and at the edge of the forest, rather than on mature trees.

Ecological indicators such as tree mortality and regeneration [116], or physiological indicators such as the dynamic of non-structural carbohydrates [117] and photosynthetic efficiency [118], may be more appropriate for assessing potential damage at ecosystem level.

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